1119-Pos Board B11

Effects of Lipids on the Structure and Function of GLIC and ELIC

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The prokaryotic pentameric ligand-gated ion channels (pLGICs), GLIC and ELIC, are excellent models for probing the mechanisms of pLGIC function. We are interested in the mechanisms by which lipids act as allosteric modulators of the prototypic pLGIC, the nicotinic acetylcholine receptor (nAChR) from *Torpedo*. In mixed phosphatidylcholine (PC) membranes such as those formed from soybean asolectin or those containing neutral and/or anionic lipids, the nAChR adopts an activatable resting conformation. In PC membranes lacking neutral and anionic lipids, the nAChR is stabilized in an uncoupled conformation that binds agonist with resting-state like affinity, but does not undergo conformational transitions. To test whether GLIC and ELIC exhibit similar lipid dependencies, we developed a protocol for reconstituting both GLIC and ELIC into model membranes composed of either soybean asolectin or PC. Both reconstituted pLGICs exhibit secondary structures similar to that of the nAChR, but both have substantially enhanced thermal stabilities, a likely requirement for the formation of diffraction quality crystals. Whereas incorporation of the nAChR into PC membranes leads to an increase in peptide hydrogen exchange relative to the nAChR in asolectin, this was not observed with GLIC and ELIC. In addition, the functional properties of both were explored by two-voltage electrode clamp electrophysiology. Our results suggest that although both GLIC and ELIC exhibit a functional sensitivity to their lipid environments, these sensitivities differ substantially from those of the *Torpedo* nAChR. These functional differences will be discussed in light of the current structures of these three pLGICs.

1120-Pos Board B12

The Release Pathway of Copper Transporting Ptype ATPases

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Cellular levels of heavy metals are carefully regulated by the PIB class of P-type ATPases in all kingdoms of life and mutations of the human members ATP7A and ATP7B are the cause of the severe Menkes' and Wilson's diseases. Recently, a crystal structure of a homologous Cu^+ ATPase from Legionella pneumophila (LpCopA), trapped in a transition state of dephosphorylation (E2Pi), suggested that copper extrusion employs an intramembranous exit site, but the release pathway remained elusive and the transmembrane (TM) domain was inferred to be occluded. However, by molecular dynamics (MD) simulations, we find that extracellular bulk water solvates the proposed exit and high-affinity ion-binding sites deep within the membrane. This view found further support by a 2.8 resolution LpCopA crystal structure trapped in the E2P state (associated with extracellular exchange in well-known PII-type ATPases such the sarcoplasmic reticulum Ca^{2+}-ATPase, SERCA) showing a similar structure of the TM-domain, and delineating the same pathway by crystal water positions. We conclude that the E2P and E2Pi states are equally open, indicating that Cu^+ ATPases couple the conformational changes associated with ion extrusion differently to dephosphorylation as compared to SERCA; in accordance with structural differences. The observed copper extrusion conduit was further validated by mutational studies and shown to involve the PIB-specific MA segment, which is absent in e.g. $Co2^+$ ATPases and thus different unloading schemes may apply within PIB-ATPases. The pathway further explains why Menkes' and Wilson's mutations at the extracellular side impair protein function and constitutes a favorable site for novel inhibitors targeting pathogens from the extracellular environment.

1121-Pos Board B13

The Disordered N-Terminus of the Plant Antenna Protein CP29 Studied by Electron Paramagnetic Resonance - Is this 100-Residue Stretch Unstructured?

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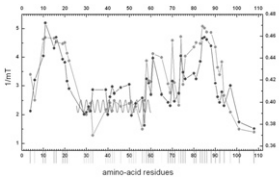
The N-terminal domain of the photosynthetic light-harvesting protein CP29, a membrane-antenna protein of PSII, is considered relevant for light-adaptation of the organism,[1] but was not resolved in the recent crystal structure of CP29.[2] We investigate the 100 amino-acids missing in that structure by site-directed spin-label EPR.[3] Mobility of the spin labels reveals that the N-terminus of CP29 is relatively structured and consists of at least five regions differing in their dynamics (Fig.). Remarkable are relatively immobilized regions, comprising the first six residues of the N-terminus and a stretch of residues from 30 to 60 with a potential α-helical region, possible attachment points to the protein surface. Distances from Double Electron-Electron Resonance (DEER or PELDOR) show defined but multiple distances, suggesting multiple conformations.



We propose that the N-terminus is flexible, but not disordered, which may be functionally important.

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1122-Pos Board B14

Interactions between Anti-HIV Antibodies and their Lipid-Embedded Epitopes Defined by EPR Spectroscopy

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A vaccine capable of stimulating protective anti-viral antibodies is needed to curtail the global AIDS epidemic. The membrane proximal ectodomain region (MPER) of the HIV envelope protein gp41 is the target of human neutralizing antibodies 4E10, 2F5, Z13e1 and 10E8. How these antibodies bind to their membrane-immersed epitopes and mediate anti-viral activity are unclear. Here, electron paramagnetic resonance (EPR) techniques were used to define the manner in which these antibodies recognize the L-shaped helix-hinge-helix MPER segment. Both 4E10 and 2F5 induce large conformational changes in the MPER relative to the membrane, and extracts buried residues from the lipids. The interaction is a stepwise and dynamic rearrangement through an apparent scoop-like movement of the antibodies' long and unique CDRH3 segments. Mutations of the CDRH3 segments reduced the ability of the antibodies to extract MPER peptides from membranes. These findings and others currently under investigation have significant implications for structure-aided vaccine design.

1123-Pos Board B15

Probing the Conformation and Dynamics of Influenza a M2 Protein using Site-Directed Spin-Label EPR Spectroscopy

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M2 is a membrane protein critical to the life cycle of influenza A. We have capitalized on the expanding body of high-resolution structural data available for the 97 amino acid M2 protein to design and interpret site-directed spin labeling electron paramagnetic resonance spectroscopy (SDSL-EPR) experiments on the conformation and dynamics of the homotetrameric M2 protein embedded in lipid bilayers. We have obtained data for three different M2 constructs (M2TM 22-46, M2TMC 23-60 and full length M2 protein) spin-labeled at multiple sites within the transmembrane and C-terminal domains. CW and pulsed EPR spectra show evidence that M2 adopts multiple conformational states in bilayers, and that cholesterol content dictates the relative populations of the states.

1124-Pos Board B16

Structure-Function Studies of Mtb Membrane Protein CrgA in Lipid Bilayer

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Tuberculosis is a deadly disease with a death toll of 1.5 million people every year and very recently an outbreak in Jacksonville, FL was devastating. All frontline antibiotics are failed for the multidrug resistant (MDR) bacilli and the key to disease control is to inhibit bacterial cell division and stabilize the bacilli in its dormant stage. Here we present the structure function studies of